Effect of Environmental Conditions and Leafhopper Gender on *Maize Chlorotic Dwarf Virus* Transmission by *Graminella nigrifrons* (Homoptera: Cicadellidae)

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ABSTRACT To determine the most economical and efficient means to maintain cultures of *Maize chlorotic dwarf virus* (MCDV) and to screen for host plant resistance to MCDV, we evaluated the effects of temperature, light intensity, daylength, atmospheric pressure, and leafhopper gender on the frequency of transmission of MCDV by *Graminella nigrifrons* Forbes (Homoptera: Cicadellidae). Female leafhoppers transmitted at higher frequencies than males under most conditions. In temperature studies, transmission rates for both male and female leafhoppers progressively increased as temperatures rose from 20 to 30°C. At high light intensities, both males and females transmitted at greater frequencies than they did at low. Similarly, longer day lengths were correlated with higher transmission rates for both sexes. No significant differences in transmission rates were observed in response to differences in atmospheric pressure. The results also showed that transmission rates under most conditions are high enough to overcome potential ambiguities caused by inoculated susceptible plants that do not become infected (disease escapes) when screening for resistance.

KEY WORDS temperature, atmospheric pressure, light

Maize chlorotic dwarf virus (MCDV) (genus *Waikavirus;* family *Sequiviridae*) causes an important stunting disease of maize, *Zea mays* L., in the United States (Gordon and Nault 1977, Gordon et al. 1981, Gingery 1988). MCDV is transmitted in a semipersistent manner by the blackfaced leafhopper, *Graminella nigrifrons* Forbes (Homoptera: Cicadellidae) and related leafhopper species (Nault et al. 1973, Nault and Madden 1988).

Maximizing the efficiency of MCDV transmission by leafhoppers is important in screening for resistance because disease escapes (susceptible plants that do not become infected) can cause susceptible plants to be falsely designated as resistant. Louie and Anderson (1993) addressed the problem of suboptimal transmission of MCDV by *G. nigrifrons* by devising a transmission method using multiple inoculations with high numbers of leafhoppers per plant. With this method, infection rates in maize inbreds increased from an average of 82% to nearly 100%. Multiple inoculations were thought to result in increased transmission, in part, by reducing the effects of environmental variables, such as temperature, light, and atmospheric pressure, on leafhopper behavior, although changes in plant susceptibility also could have contributed to variability in transmission rates. However, raising the numbers of insects required for the method is both labor-intensive and expensive and the increased time required slows progress. A better understanding of how environmental conditions influence transmission of MCDV by *G. nigrifrons* might reveal how to achieve high transmission rates and reduce the numbers of insects required for experiments.

Insect behavior, including virus transmission, can be affected by environmental conditions such as temperature, atmospheric pressure, light, relative humidity, precipitation, and wind (Wellington 1957, Ling and Tiongco 1979, Anderson et al. 1993). The experiments described here were designed to determine the effect of differences in temperature, light intensity, light duration, and atmospheric pressure on MCDV transmission efficiency by *G. nigrifrons.*

Materials and Methods

MCDV Isolates and Leafhopper Rearing. Colonies of *G. nigrifrons* were maintained in greenhouse and growth chamber rearing facilities in cages containing oats, *Avena sativa* L., or maize inbred Oh28 as described by Guthrie et al. (1982). The MCDV isolate used was the severe strain described by Hunt et al.

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(1988) because it produces bright diagnostic tertiary vein chlorosis symptoms (Gordon and Nault 1977). It was maintained in inbred Oh28 maize or Seneca Chief sweet corn by serial transfer by using *G. nigrifrons.*

Virus Acquisition and Inoculation. Three to 4 d before use, nonviruliferous adult leafhoppers were anesthetized with CO_2, sexed, and held on healthy maize in a growth chamber at 24°C, 14-h daylength, and 38 W/m^2 light intensity. Before beginning an experiment, leafhoppers were given a 1-d acclimation period at the temperature and lighting conditions to be used for acquisition and inoculation. For acquisition, leafhoppers were caged for a 24-h acquisition access period (AAP) on source plants (MCDV-infected Oh28 maize that had been inoculated by viruliferous leafhoppers 18–20 d before) in ventilated polycarbonate tubes (30.5 cm in length by 7.6 cm in diameter). They were then transferred to test plant seedlings (Seneca Chief sweet corn planted 4 d before) at a density of two leafhoppers per plant for a 48-h inoculation access period (IAP). Test plants (2–5 cm in height) were individually enclosed in ventilated plastic (butyrate) cages (15.2 cm in length by 3.8 cm in diameter). After inoculation, leafhoppers were removed and the plants fumigated with resmethrin to kill any remaining insects. The plants were observed for tertiary vein chlorosis after 14 d. Fourteen days was selected based on results from other, unrelated experiments in which plants were examined for symptoms for periods up to 4 wk. In no case did symptom onset begin later than 14 d after inoculation. The individual transmission rate (p) was calculated by the formula $p = 1 - (1 - I)^{1/k}$, in which I is the proportion of infected plants, and k is the number of insects placed per plant (Swallow 1985). In these experiments, $k = 2$. Differences in means were assessed using a two-sample t-test assuming unequal variances.

Environmental Data Measurements. In the greenhouse, temperature measurements were taken with a Campbell model CS500 probe (Campbell Scientific, North Logan, UT). Light intensities were measured with an Eppley model 8-48 pyranometer (The Eppley Laboratory, Newport, RI) at bench height (91.4 cm; 36 in.) outside of cages. Reductions in light intensity while plants were caged for leafhopper acquisition and inoculation were not determined. Atmospheric pressure was measured with a model 270 Setra pressure transducer (Setra Systems, Inc., Acton, MA). The instruments were connected to a Campbell model 21X datalogger, and the data were averaged and recorded every minute.

Correlation of Environmental Variables and Leafhopper Transmission of MCDV. Initial transmission tests were conducted each week for 50 wk over a period of ≈14 mo in a greenhouse set at 25°C with no supplemental lighting. Because of seasonal ambient influences, temperatures varied from 21 to 29°C over the period. Every week, 50 plants were inoculated with 100 male leafhoppers and another 50 plants were inoculated with 100 female leafhoppers as described above. Separately for each gender, Pearson's correlation coefficients (r) were calculated to determine whether changes in transmission rates could be correlated with changes in light intensity or atmospheric pressure during acquisition or inoculation, or with daylength (sunrise to sunset). After the greenhouse studies, controlled experiments in growth chambers were conducted to separately assess the influence of environmental variables.

Effect of Temperature on Leafhopper Transmission of MCDV. For temperature studies, all steps from acquisition through symptom recording were done at the same temperature in Rheem model CEC 255-6 growth chambers (Rheem Scientific Products, Asheville, NC) at 20, 25, or 30°C with a 14-h daylength and a light intensity of 38 W/m^2. In each of 10 replicates (five for males and five for females), 50 plants (100 leafhoppers) were used at each temperature tested.

Effect of Daylength and Light Intensity on Leafhopper Transmission of MCDV. Experiments were done in EGC model M-48 walk-in growth chambers (Environmental Growth Chambers, Chagrin Falls, OH). For each light regime, 50 plants (100 leafhoppers) were used, and all steps from acquisition through recording of symptoms were done at the same light conditions. Light regimes tested were high light (250–280 W/m^2) and low light (73–75 W/m^2) each at 14- and 9-h daylengths. The experiment was replicated six times.

Effect of Atmospheric Pressure on Leafhopper Transmission of MCDV. Pressure studies were done in both the Rheem and EGC growth chambers held at 24.0 ± 0.1°C with a 14-h daylength. The light intensities were 38 W/m^2 in the Rheem growth chamber and 250–280 W/m^2 in the EGC growth chamber. Atmospheric pressure (mmHg) was recorded hourly and averaged over the AAP and IAP. Each trial consisted of 50 test plants (100 leafhoppers). Ten trials for males and 10 for females were performed over a period of 10 wk.

Statistical Analysis. Pearson's correlation coefficients were determined and stepwise regression analysis was applied to results from the effects of environmental variables on transmission experiment. Results from all other experiments were subjected to analysis of variance (ANOVA-GLM).

Results

Effect of Environmental Variables on MCDV Transmission in the Greenhouse. Scatter plots of individual rates of transmission of male and female leafhoppers and various environmental variables are shown in Figs. 1 and 2, and the corresponding correlations and probabilities that the correlations were significant are listed in Table 1. For both males and females, transmission rates were significantly correlated with light intensity during acquisition (Figs. 1A and 2A), light intensity during inoculation (Figs. 1B and 2B), and daylength (Figs. 1C and 2C). The correlations between transmission rate and atmospheric pressure during either acquisition (Figs. 1D and 2D) or inoculation (Figs. 1E and 2E) were not significant.

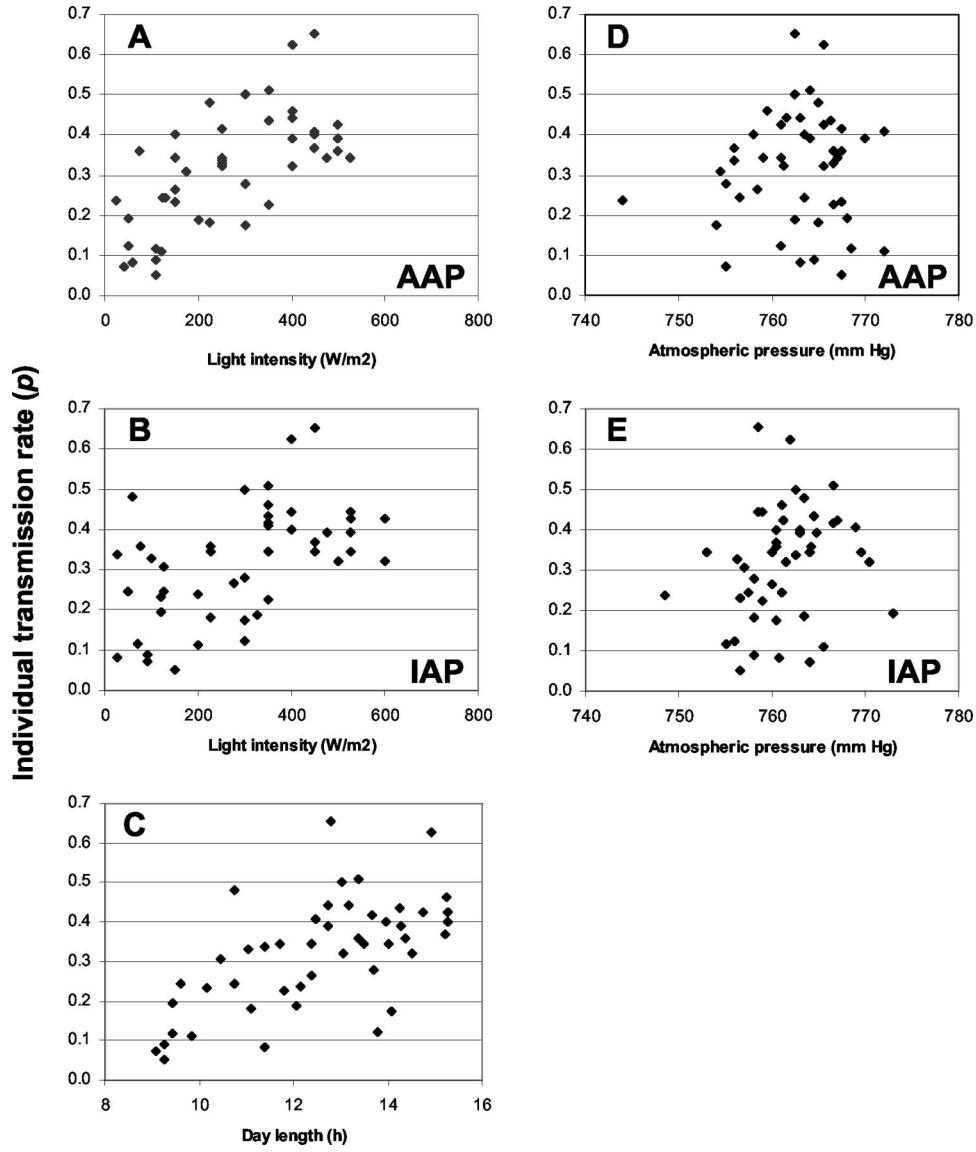

Fig. 1. Scatter plots showing the relationship of light intensity (A and B), daylength (C), and atmospheric pressure (D and E) to transmission of MCDV by male *G. nigrifrons*. Light intensity and atmospheric pressure values represent the average of readings taken at the beginning and end of 1-h AAPs on MCDV-infected Oh28 maize plants and 2-h IAPs on Seneca Chief sweet corn seedlings. Daylengths were recorded at the beginning of each trial. Each point represents the mean individual transmission efficiency calculated for one of 50 separate trials done over a 14-mo period. Each trial consisted of 50 test plants inoculated with two leafhoppers each.

Stepwise regression analysis revealed that light intensity during AAP, light intensity during IAP, and daylength could predict the individual transmission rate ($R^2 = 53.9\%$). The regression equation was as follows: $p = -0.19 + 0.00049(\text{light}_{\text{AAP}}) - 0.00020(\text{light}_{\text{IAP}}) + 0.036(\text{DayL})$, where p is individual transmission rate, $\text{light}_{\text{AAP}}$ is light intensity (W/m^2) during AAP, $\text{light}_{\text{IAP}}$ is light intensity (W/m^2) during IAP, and DayL is daylength in hours.

Based on the above-mentioned correlations, the effects of leafhopper sex, light intensity, daylength, and atmospheric pressure on MCDV transmission by *G. nigrifrons* were further examined under controlled conditions in growth chambers. The effects of temperature on transmission also were tested.

Effect of Temperature on MCDV Transmission in Growth Chambers. ANOVA revealed significant effects on individual transmission rates for sex and temperature (Fig. 3). Females transmitted at significantly higher rates than males ($F = 19.00, P = 0.0002$), and both males and females transmitted at higher rates with rising temperatures between 20 and 30°C ($F =$

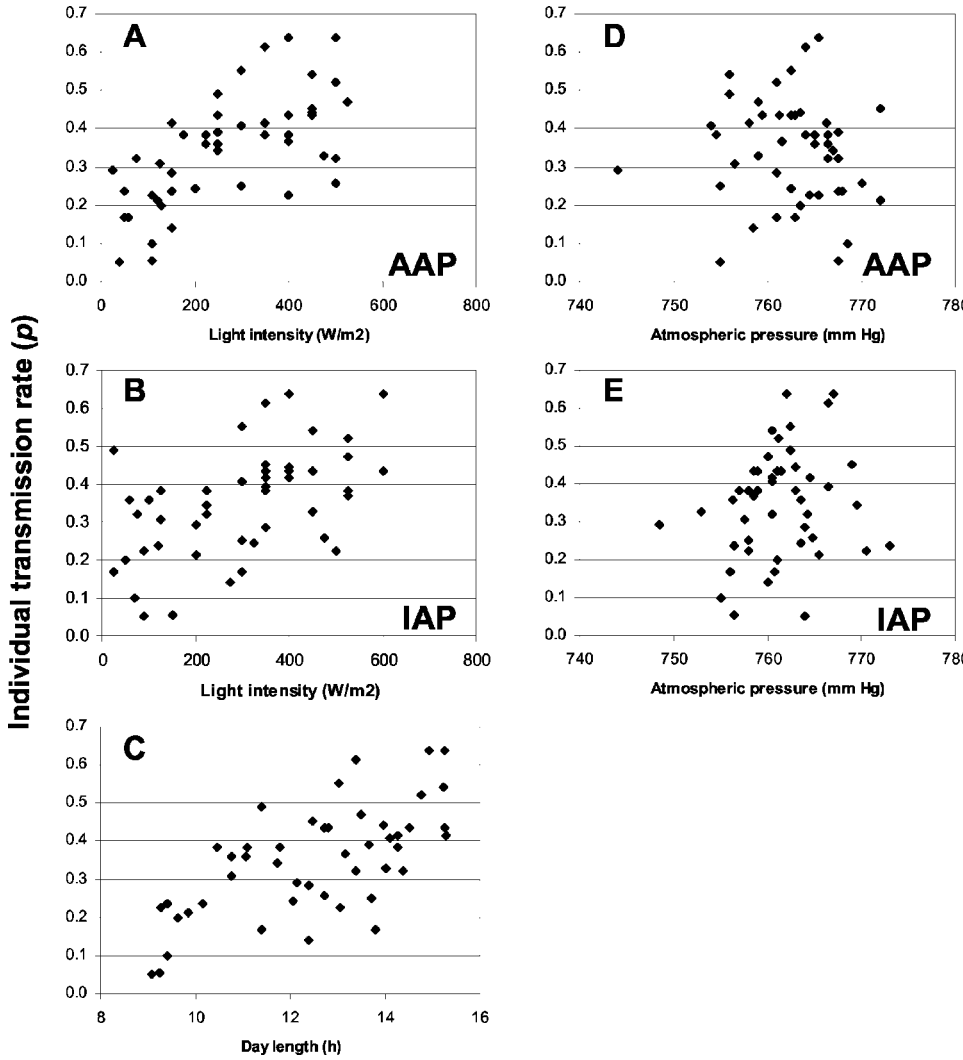

Fig. 2. Scatter plots showing the relationship of light intensity (A and B), daylength (C), and atmospheric pressure (D and E) to transmission of MCDV by female *G. nigrifrons*. Light intensity and atmospheric pressure values represent the average of readings taken at the beginning and end of 1-h AAPs on MCDV-infected Oh28 maize plants and 2-h IAPs on Seneca Chief sweet corn seedlings. Daylengths were recorded at the beginning of each trial. Each point represents the mean individual transmission efficiency calculated for one of 50 separate trials done over a 14-mo period. Each trial consisted of 50 test plants inoculated with two leafhoppers each.

8.70, $P = 0.0015$). The interaction between sex and temperature was not significant ($F = 1.85$, $P = 0.1798$).

Effect of Light Intensity and Daylength on MCDV Transmission in Growth Chambers. ANOVA revealed significant effects on individual transmission rates for sex ($F = 19.40$, $P < 0.0001$) and light intensity ($F = 78.57$, $P < 0.0001$) as well as their interaction ($F = 21.23$, $P < 0.0001$) (Fig. 4). Females transmitted at significantly higher rates than males at high light intensity, but they were not significantly different and even lower than males at low light intensity; thus, the significant interaction. There were also significant effects on transmission for daylength ($F = 31.02$, $P < 0.0001$) and the interaction of daylength and sex ($F = 8.08$, $P = 0.007$). The effects of daylength were most apparent at low light intensities for both genders. There was no significant interaction between light intensity and daylength ($F = 0.23$, $P = 0.6340$).

Effect of Atmospheric Pressure on Transmission Efficiency in Growth Chambers. There were no significant correlations between atmospheric pressure and transmission of MCDV by either male or female *G. nigrifrons* ($r = -0.260$–0.078). Atmospheric pressure averages ranged from 760 to 772 mmHg over the 10-wk course of the experiments.

Discussion

The rationale for conducting these experiments was to determine how the environmental parameters, tem-

Table 1. Correlation of environmental variables with efficiency of transmission of MCDV by *G. nigrifrons* in a greenhouse

Environmental parameter	Leafhopper sex	Pearson's correlation coefficient (r)	P^a	Significant	Corresponding figure
Light intensity during AAP	Male	0.670	<0.001	Yes	1A
	Female	0.664	<0.001	Yes	2A
Light intensity during IAP	Male	0.531	<0.001	Yes	1B
	Female	0.538	<0.001	Yes	2B
Daylength	Male	0.623	<0.001	Yes	1C
	Female	0.669	<0.001	Yes	2C
Atmospheric pressure during AAP	Male	0.031	0.831	No	1D
	Female	0.088	0.543	No	2D
Atmospheric pressure during IAP	Male	0.226	0.115	No	1E
	Female	0.184	0.201	No	2E

[a] Probability that r does not represent a significant correlation determined by a t-test where $t = r/s_r$ and s_r is the standard error of r.

perature, atmospheric pressure, light intensity, and daylength affect transmission of MCDV by *G. nigrifrons.* Differences in transmission due to leafhopper gender also were investigated. The goal was to identify conditions conducive to high transmission rates so that 1) viruses could be more easily and economically maintained by serial leafhopper transmission; and 2) host plant resistance could be reliably assessed with the fewest numbers of insects. The results showed that transmission rates were significantly influenced by changes in temperature, light intensity, and daylength, but not by differences in atmospheric pressure.

Transmission rates by both females and males increased with increasing temperature from 20 to 30°C, which encompasses temperatures likely to be encountered in most growth chambers and greenhouses. In another study of the influence of temperature on waikavirus transmission, Ling and Tiongco (1979) observed a similar increase in transmission efficiencies with increasing temperature for the rice green leafhopper, *Nephotettix virescens* (Distant), and *Rice tungro virus.*

Light intensity and daylength also affected transmission efficiencies. Rates were generally greater under high light conditions compared with low. Daylength was of little importance under high light conditions. Under low light, daylength was an important factor. Decreased rates of transmission at lower light levels, lower temperatures, and shorter days may explain, in part, why MCDV transmission rates are reduced in winter, particularly in the greenhouse without supplemental lighting (R.J.A., unpublished observation). Light intensities generated by the growth chambers in these experiments were 30–50% of the intensity of direct sunlight at noon in summer in the Midwest.

Females transmitted MCDV more efficiently than did males under most conditions tested, but the differences were not great enough to warrant sexing leafhoppers before MCDV transmission tests. Atmospheric pressure levels had little or no effect on transmission and that is fortunate because pressure is difficult and expensive to control and manipulate. This is consistent with a previous study showing no effect of atmospheric pressure on MCDV transmission rates by *G. nigrifrons* males or females (Anderson et al. 1993).

The transmission rates observed at high light levels and 25–30°C are adequate for efficient serial transmis-

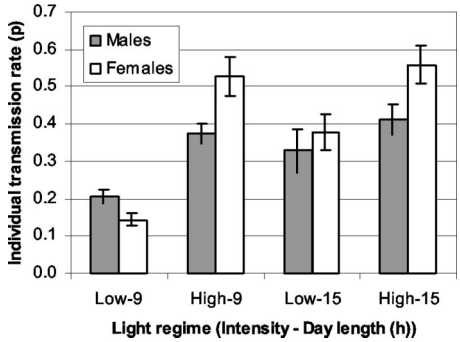

Fig. 3. Effect of temperature on leafhopper transmission of MCDV. Male or female *G. nigrifrons* leafhoppers were given a 24-h AAP on MCDV-infected maize followed by a 48-h IAP on maize seedlings at the temperatures indicated. Bars, standard errors of the mean of five replicates.

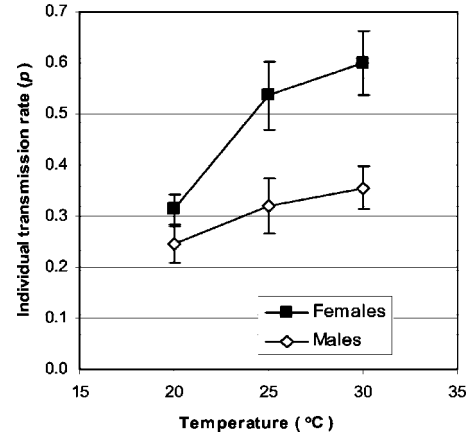

Fig. 4. Effect of light intensity and daylength on transmission of MCDV by *G. nigrifrons.* Leafhoppers were given a 24-h AAP on MCDV-infected maize followed by a 48-h IAP on maize seedlings at the light conditions indicated (low, 73–75 W/m^2; high, 250–280 W/m^2). Bars, standard errors of the mean of six replicates.

sion of MCDV cultures and for effective screening for resistance. For example, the equation $p = 1 - (1 - I)^{1/k}$ predicts that the rate of transmission to test plants by using 10 leafhoppers per plant would be 99% or greater for individual transmission rates above 0.37, a rate commonly observed during this study. At such high overall transmission rates, disease escapes would not significantly interfere with resistance screening trials. However, if individual transmission rates dropped to 0.15 or less, as might be observed during cool periods in the winter, the overall rate of transmission using 10 leafhoppers per plant would be 80% or less, and disease escapes may confound the results of resistance screening. In such cases, the multiple inoculation method of Louie and Anderson (1993) could be used to raise the overall infection rate to acceptable levels, the number of leafhoppers could be increased, or, as shown in this study, rates could be increased by increasing the temperature or light intensity.

Accurately assessing the quantitative effect of environmental variables on insect transmission of viruses is inherently difficult because of likely interrelationships among the variables and the effects of unknown factors on leafhopper behavior. Nevertheless, results of these experiments show that environmental and experimental conditions can significantly affect the efficiency of leafhopper transmission of MCDV, and presumably other plant viruses as well. The results also show that MCDV transmission rates by *G. nigrifrons* under most conditions are high enough so that relatively few inoculated susceptible plants will escape infection and by erroneously scored as resistant.

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